Chemistry of Natural Compounds, Vol. 35, No. 4, 1999

SYNTHESIS OF METALLOPROTEIN COMPLEXES AND STUDY OF THE INFLUENCE OF METAL IONS ON THE STRUCTURE OF A CARRIER PROTEIN

A. A. Abduvaliev,[b] O. Ya. Ikramov,[b] and V. P. Tatarskii[a]

UDC 547.455.9.057;616-006

The results are given of an investigation on the synthesis of protein conjugates with a number of metals differing by their valence and electronic state, and on their immunocompetence.

The wide distribution of metals and their compounds in everyday life and professional activity, including their use as medicinal components, has led to an increase in allergic reactions. The mechanism of the action of these substances on the human organism and the pathways of he development of disease have been studied inadequately.

The biological activity of metals is connected with their capacity for damaging the cell membrane, for increasing the permeability of barriers, for being bound with proteins, and for blocking many enzyme systems, which, taken together, lead to a disturbance of intracellular metabolic processes. At the same time, some complexes of metals with natural biopolymers may prove useful in elucidating the role of their structural-conformational changes in various pathologies, including the malignant degeneration of cells. In view of this, we have attempted to synthesize metalloprotein complexes and to study the influence of metal ions on the structure of the biopolymeric molecule.

EXPERIMENTAL

In the experiments we used bovine serum albumin (BSA) and rabbit serum albumin (RSA) produced by the Nikhol experimental factory (Tashkent) and chemical compounds of metals — CsCl, W_2O_3, $Pb(NO_3)_2$ and $InSO_4$ — of ch.d.a [pure for analysis] grade.

Conjugation was conducted by a standard method [1]. The conjugates were freed from an excess of chemical reagents and metal ions by dialysis. The presence of cesium and lead in the conjugates was determined by the appropriate procedures [2, 3].

Mice of the Balb line were immunized with the protein-metal complexes. Rabbits of the Seryi velikan ["Gray giant"] breed were also immunized with the conjugate RSA-Cs.

Results — precipitations were carried out by the generally adopted methods of Ouchterlony immunoprecipitation in agar-agar and by immunoenzyme assay (IEA) (sandwich method using a conjugate of peroxidase with antibody to mouse immunoglobulins). As antigens we used 1% solutions of salts of the corresponding metals and their conjugates with protein. In the IEA method, optical densities were determined on a Multiscan instrument.

Electrophoresis was conducted by a generally adopted method in 12.5% polyacrylamide gel (PAAG). Protein was determined by Bradford's method with calibration in relation to albumin.

We synthesized protein—metal conjugates of the following types: RSA-Cs, BSA-Cs, BSA-W, BSA-Pb, and BSA-In. In the RSA-Cs, BSA-Cs, and BSA-Pb conjugates we determined the presence of cesium and lead ions by qualitative reactions. Rabbits were immunized with the RSA-Cs conjugate, and mice with BSA-Cs, BSA-W, BSA-Pb, and BSA-In.

---

a) Institute of Oncology and Radiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent; b) Nikhol Experimental Factory, Institute Oncology and Radiology, Academy of Sciences, Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 510—511, July-August, 1999. Original article submitted March 15, 1999.

0009-3130/99/3504-0455$22.00

After the antisera had been taken, precipitation was carried out in agar-agar against the protein—metal conjugates, metal salts, and native BSA (in the case of RSA-Cs — RSA) with dilution steps of 1:10. No true precipitation was detected in the case of any of the above compounds. In view of this, we conducted IEA using antibodies against mouse immunoglobulins and the conjugates BSA-Cs, BSA-W, BSA-Pb, and BSA-In. The IEA results showed the absence of a titer of the antibodies both to the conjugates and to native BSA. We then immunized mice with native BSA from the serum used in the experiments with metals. The results showed a high titer of the antibodies to native BSA (1:2500).

For an indirect determination of the structure of the protein in the BSA-Cs conjugate we used electrophoresis in 12.5% PAAG The results showed that after conjugation with a metal the protein did not form conglomerates and did not undergo degradation; moreover, the mobility of the RSA-Cs did not differ from that of the native RSA.

Thus, it may be concluded that the introduction of protein—metal conjugates into the animal organism leads to disturbances connected with the immune response in the reaction system through class IgE immunoglobulins. This hypothesis requires additional investigations.

REFERENCES

1. A. A. Nikitenko et al., in: *Questions of Infectious Pathology* [in Russian], Moscow (1973), p. 258.
2. V. E. Payushchev and B. D. Stepin, *The Analytical Chemistry of Rubidium and Cesium* [in Russian], Moscow (1975), p. 42.
3. N. G. Polyanskii, *Lead* [in Russian], Moscow (1986), p. 46.